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(12) # EUROPEAN PATENT SPECIFICATION

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(54) **Covalent attachment of anticoagulants and the like onto biomaterials.**

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EP-A- 0 028 122
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DE-A- 3 109 141
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GB-A- 1 583 008

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Description

The invention relates to a substrate of a medical device with a blood-compatible surface having a physiologically active substance which has an inhibitory effect on the formation of blood clots or is capable of breaking down blood clots formed, immobilized onto the surface via a functional group of a compound which may act as a spacer, covalently bonded to the surface.

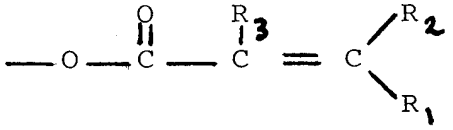
As is known, various attempts have been made to improve the blood compatibility of various kinds of biomaterials by immobilizing on their surface heparin or heparin analogues. Thus it is known from United States Letters Patent No. 4,526,714 to render the surface of a substrate biocompatible by coating it with a conjugate of heparin, heparinous material or heparin analogues and a protein, the conjugate being formed by coupling that is carried out in the presence of 1-ethyl-3-dimethyl-aminopropyl carbodiimide (EDC) and the like as a coupling agent. The conjugate is attached to the substrate surface at the sites of the surface where free functional groups suitable for binding to the conjugate are present. In order to effect the coupling needed to form this known conjugate, these free functional groups on the substrate surface are provided as free amino groups.

For the blood compatibility of this prior art substrate surface to increase, the degree of coverage of the surface with the conjugate must be increased, which, for all practical purposes, means that the substrate surface should have a similarly large number of free functional groups available which are suitable for bonding. Since the surface of a substrate often does not have free functional groups such as amino groups, these groups should first be liberated from the substrate material. This can be effected, for example, by chemical means, which is then accompanied by an attack on, i.e. modification of or damage to, the substrate surface. This damage is, of course, more severe as the number of free functional groups that must be provided is increased.

From DE-A-3 109 141 it is known to render a substrate surface of a medical device as a substrate to have antithrombotic properties through covalently coupling in the presence of a coupling agent, such as a water soluble carbodiimide, of a physiological active material such as heparin (HEP) to said substrate surface by means of a so-called "spacer" molecule having a specified length of 3-150 Å. The spacer is defined as being a monomer, dimer, trimer and/or oligomer with each of its both ends carrying a functional group. The spacer molecule with one end is covalently coupled to the substrate surface and with its other end to the physiological active material. Consequently the ratio of (HEP molecules immobilized onto the substrate surface/the number of bonding sites on the substrate surface) is 1.

Within the context of providing a substrate surface having biocompatible properties it is disclosed in FR-A-2 187 849 to coat by a graft reaction a chemically active compound selected from a polymer or a copolymer onto an inert material as the substrate. Following the coating of the substrate a molecule of a compound having said biological properties such as heparin is chemically coupled to the coating. Now, providing a coating onto a substrate surface consequently means a complete modification of the substrate surface.

It is furthermore known from EP-A-0 046 828 to impart antithrombotic activity to a biomedical material by treating its surface that makes contact with blood with an antithrombotic agent such as a HEP derivative wherein at least 0,5%, preferably 1-80% of the entire hydroxyl group content of heparin have been esterified by means of an acid providing ester groups of the formula



Preferably $R_1 = R_2 = R_3 = H$ or $R_1 = R_2 = H$ and $R_3 = CH_3$ which means that the unsaturated acid used for esterification is acrylic acid or methacrylic acid, respectively. In practice this means that every HEP derivate ultimately bonded to the substrate surface comprises at least one bonding site and so the ratio of (HEP molecules bonded to the substrate surface/the number of bonding sites) is 1 or less. Consequently in increasing the blood compatibility of this prior art substrate surface by increasing the degree of coverage of the surface with HEP molecules ultimately bonded to the surface may even lead to an increase of the number of bonding sites on the substrate surface per HEP molecule, hence to an increased damage to said surface.

It is an object of the invention to provide a substrate of a medical device having a blood-compatible surface, in which a large and typically controllable amount of a physiologically active substance is connected via anchoring sites available on the surface of a substrate while avoiding or causing disproportionately little damage to the substrate surface.

These and other objects, features and advantages of this invention will be clearly understood through a consideration of the following detailed description.

According to the invention the substrate as cited in the opening paragraph herein above said compound is a polyacid polymer having many carboxylic acid groups as the functional groups to which said physiological active substance is directly or indirectly bonded and said polyacid polymer is covalently bonded to the uncoated surface of the substrate.

The invention is based on the insight that, owing to the introduction of a polyacid polymer in the link chain of the physiologically active substance to the substrate surface, the carboxyl groups of each polyacid polymer molecule provide a large number of free functional groups not belonging to the original substrate surface, which can serve as many bonding sites for anchoring the physiologically active substance. Accordingly, although only a small number of functional groups, such as amino groups, are introduced to, or liberated on, the original substrate surface, the number of potential bonding sites for the ultimate substance affecting the coagulation of blood is increased dramatically by the numerous multiple free carboxylic acid functional groups provided by the polyacid polymer.

In GB-A-1 583 008 a process is disclosed comprising the step of treating a polymere material such as polyacrylic acid with a solution of a synthetic fibrinolytic compound thereby to cause said compound to covalently bond to the polymeric material to provide it with antithrombogenic properties, it is true. However, according to the prior art proces it is the polymeric material such as polyacrylic acid itself which is to be made antithrombogenic. In contradiction thereto according to the invention the polyacid polymer such as polyacrylic acid constitutes only part of a chain ultimately connecting a fibrinolytic compound such as heparin to a substrate surface resulting in said surface obtaining antithrombotic properties.

In the course of this description, reference will be made to the accompanying Figure, which is a formulae sheet exemplifying a typical reaction scheme according to the present invention.

Any substrate having a surface that bonds with a polyacid formed or to be formed is suitable according to the invention. One example is a substrate of a material in which the polyacid polymer is directly bonded to the substrate surface and is formed by polymerizing a COOH-group containing monomer from the substrate surface to the polyacid polymer, or in which the polyacid polymer, preferably with a double bond at the chain terminal, is directly (continued by page 4, line 1 ff. of the original specification). attached to the substrate surface by this terminal by means of a graft reaction. Another example is a substrate made from a material suitable for liberating functional groups from the substrate surface thereof. Examples of substrate materials belonging to this category are those having free amino, isocyanate, carboxyl, and/or alcohol groups available as functional groups, or that are capable of providing such groups. The polyacid can be directly coupled to the functional group, for example, if this group is an amino group, or coupling can be achieved after first chemically modifying the functional group, for example, in the case of a carboxyl group, which is first modified by a low-molecular weight diamine before being reacted with the polyacid.

A polyacid polymer suitable for the purposes of the invention is preferably a polyacid polymer that is water-soluble. This type of polyacid polymer may contain an aliphatic main chain to which carboxyl groups are attached, optionally via a side chain. An example of this type of polyacid polymer are polyacrylic acids which may be built up from numerous monomeric units, for example between 1000 and 10,000 units, and in which each monomeric unit accordingly contains one carboxyl group. Polymethacrylic acids are likewise suitable. Also suitable is a polyacid polymer having a non-aliphatic main chain, for example, polyaspartic acid and polyglutamic acid. Furthermore, a polymer can be used that exhibits two or more carboxyl groups per monomer unit. In addition, the substrate according to the present invention may contain a polyacid polymer which is cross-linked.

The physiologically active substance to be used according to the invention is one having an inhibitory effect on the formation of blood clots or has the capability of breaking down blood blots formed. It may be a substance having an anticoagulant effect, a substance having a fibrinolytic activity, a substance having a blood platelet aggregation inhibiting effect and/or a blood platelet adhesion inhibiting effect. For example, heparin, a heparinoid, a prostaglandin, urokinase, streptokinase or combinations thereof may be used. Heparinous materials typically are used.

Although not necessary, it is advantageous for the substance with an inhibitory effect on the formation of blood clots or capable of breaking down the blood clots formed to be connected via a spacer compound to the polyacid, which itself, for that matter, also functions as a spacer. The spacer compound may be a compound containing more than one NH_2 group, for example, Jeffamine® which is a polyethylene oxide containing terminal amino groups. Another suitable spacer compound is, for example, a protein.

The invention also relates to a method of making the substrate with a blood compatible surface. Included is a pre-stage or first step, in which a number of functional groups are liberated from the substrate surface, or are introduced into the sub-

strate surface. In a first stage or second step, the functional group connected to the substrate surface is coupled to a polyacid, polymer and thereafter in a second stage or third step, the physiologically active substance is coupled to the polyacid polymer via a spacer compound containing more than one amino group.

The polyacid polymer may be directly attached to the substrate surface owing to its being formed by polymerizing a monomer from the substrate surface that contains at least one COOH group or owing to having the polyacid polymer attached to the substrate surface via a graft reaction, in which case a polyacid polymer can be used which contains a C = C double bond. It is also possible to use a polyacid polymer containing two or more carboxyl groups per monomer unit, for example, polymaleic acid or an activated precursor of the polyacid polymer, a particular example being a polyacid anhydride.

With reference to the reaction scheme indicated on the accompanying sheet of formulae shown in the Figure, the invention and especially the method according to the invention are exemplified by reference thereto. Details of this example of the present invention are as follows.

In the example shown in the Figure, a substrate material capable of providing amino groups as free functional groups is used. During a preliminary reaction stage, free amino groups will be liberated. If the substrate material already exhibits free amino groups or the like, this preliminary reaction stage could be omitted. If the substrate material is, for example, a polyetherurethane, the surface thereof can be treated chemically in the manner illustrated by reaction (a) in the Figure. For example, such a substrate may be subjected to hydrolysis for about thirty minutes in a 3M solution of NaOH in water at a temperature of about 60° C. In this illustrated reaction, urethane bonds are broken, and amino and hydroxyl groups are formed.

Other methods of introducing free amino groups into the substrate surface are also suitable. For example, free amino groups can be introduced physically by means of so-called "plasma glow discharge", according to which method radicals are formed at the substrate surface of such an elected type that these provide, for example, free NH_2 groups in interaction with suitably selected compounds in the gaseous phase.

The free amino groups formed are subsequently covalently bonded to a polyacid polymer, for example, polyacrylic acid in which n = 1000 as illustrated in reaction stage (A) in the Figure. Based on a substrate surface containing free amino groups, the coupling with the polyacrylic acid can be effectively carried out in the presence of a coupling agent, for example, a carbodiimide. Preferably, a water-soluble carbodiimide is used, because water is not corrosive relative to the substrate material. The function of the coupling agent such as a carbodiimide in the coupling reaction is that in which a portion of the free carboxyl groups of the polyacid are activated by the carbodiimide, and these activated carboxyl groups in turn react with a free NH_2 group of the substrate.

The coupling product of the free amino group of the substrate with polyacrylic acid, illustrated in reaction stage (B) of the Figure, is subsequently brought into a form in which it can serve as a basis for the ultimate immobilization of a physiologically active substance, for example, heparin, to the substrate surface. For this purpose, the free carboxyl groups of the polyacid polymer are coupled to the polyethylene oxide (PEO) with terminal amino groups Jeffamine® as illustrated in reaction stage (B) in the Figure.

In addition to serving as a supplier of an attachment site for the ultimate physiologically active substance to be immobilized, the amino-terminated polyethylene oxide having more than one amino group is active as a so-called "spacer" group. It is known that physiologically active substances, such as heparin, function better in an environment in which they are relatively more mobile than one in which they are immobilized, such as being attached to a substrate. A direct coupling of heparin or the like to the substrate surface immobilizes same to a great extent, as a result of which its physiological activity is reduced. Accordingly, the polyethylene oxide functions as a "spacer" group to provide additional space between the physiologically active substance and the substrate surface to which it is ultimately bonded.

Inasmuch as the reaction indicated in reaction stage (B) of the Figure is of a type along the lines of reaction stage (A), the coupling according to reaction stage (B) can also be effectively carried out in the presence of a coupling agent such as a carbodiimide when desired.

In a last stage which is illustrated in the Figure as reaction stage (C), which is in accordance with the preferred embodiment, a heparin (HEP) containing a terminal aldehyde group is attached. The aldehyde group forms a Schiff's base with the free amino group of the amino-terminated polyethylene oxide. This Schiff's base can be reduced to form a stable secondary amine by means of sodium cyanoborohydride. The reaction product obtained by following the reaction scheme illustrated in the Figure is the reaction product having Formulae 1, which is bonded to the substrate surface.

It is also possible to vary the reaction sequence. For example, one could first react a heparinous material containing a terminal aldehyde group with an excess of non-bonded amino-termi-

nated polyethylene oxide in the presence of a reducing agent, such as sodium cyanoborohydride. Subsequently, the reaction product thus obtained is coupled via the free amino group of the polyethylene oxide moiety of the molecules of the reaction product to the polyacid polymer whose carboxylic acid groups have been pre-activated by means of, for example, a water-soluble carbodiimide.

As stated before, in addition to heparin, other blood coagulation affecting substances may be bonded to the reaction product having Formula 1. Thus, for example, when a polyacrylic acid with 1000 monomer units is used, it is possible to realize such a distribution that, in the reaction product having Formula 1, a molar ratio of 75% heparin and 25% other physiologically active substances is possible, for example.

By rendering a substrate surface biocompatible in accordance with the present invention, various advantages can be realized. Due to the use of the polyacid polymer, as much of a physiologically active substance, such as a blood coagulation affecting substance, for example heparin, can be bonded to the substrate as is considered desirable. The extent or relative amount of physiologically active substance that can be bonded to a substrate, for example a catheter, will generally depend on the molecular weight of the polyacid polymer and can be generally controlled to assure blood compatibility of the substrate.

In addition, as is exemplified in reaction stage (A) of the Figure, the use of a polyacid polymer and the reaction for liberating functional groups, for example to provide amino groups, does not necessarily have a particularly high efficiency. In the case of hydrolysis of a polyetherurethane surface, this means, for all practical purposes, that it is not necessary to split a large amount of chains, so that surface damage can be reduced, while the introduction of functional groups at the substrate surface only needs to take place on a limited scale. Furthermore, instead of effecting a direct coupling of the spacer compound to the substrate surface, the use of the polyacid permits an increase in the mobility of the coupled blood coagulation affecting substrate such as heparinous material. This mobility is of importance for the anti-coagulant effect of the bonded physiologically active substance or substances.

It will be understood that the embodiments of the present invention which have been described are illustrative of some of the applications of the principles of the present invention. Numerous modifications may be made by those skilled in the art without departing from the true spirit and scope of the invention.

Claims

1. A substrate of a medical device with a blood-compatible surface having a physiologically active substance which has an inhibitory effect on the formation of blood clots or is capable of breaking down blood clots formed, immobilized onto the surface via a functional group of a compound which may act as a spacer, covalently bonded to the surface, **characterized** in that said compound is a polyacid polymer having many carboxylic acid groups as the functional groups to which said physiological active substance is directly or indirectly bonded and said polyacid polymer is covalently bonded to the uncoated surface of the substrate.

2. The substrate as claimed in claim 1, wherein said polyacid polymer is polyacrylic acid or polymethacrylic acid.

3. The substrate as claimed in claim 2, wherein said polyacid polymer is a polymer containing from between about 1000 and 10.000 monomeric units.

4. The substrate as claimed in claim 1, wherein said polyacid polymer is polyaspartic acid.

5. The substrate as claimed in claim 1, wherein said polyacid polymer is polyglutamic acid.

6. The substrate as claimed in claim 1, wherein said polyacid polymer is cross-linked.

Patentansprüche

1. Ein Substrat einer medizinischen Vorrichtung mit einer blutkompatiblen Oberfläche mit einer physiologisch aktiven Substanz, welche eine inhibitorische Wirkung auf die Bildung von Blutgerinnseln hat oder welche in der Lage ist, gebildete Blutgerinnsel abzubauen, immobilisiert auf der Oberfläche über eine funktionelle Gruppe einer Verbindung, welche als ein Spacer wirken kann, kovalent gebunden an die Oberfläche,

   dadurch gekennzeichnet, daß

   die Verbindung ein Polysäurepolymer ist mit vielen Carboxylsäuregruppen als den funktionellen Gruppen, an welche die physiologisch aktive Substanz direkt oder indirekt gebunden ist und das Polysäurepolymer kovalent an die unbeschichtete Oberfläche des Substrates gebunden ist.

2. Das Substrat nach Anspruch 1, worin das Polysäurepolymer Polyacrylsäure oder Polymethacrylsäure ist.

3. Das Substrat nach Anspruch 2, worin das Polysäurepolymer ein Polymer ist, welches zwischen etwa 1000 und 10000 monomere Einheiten enthält.

4. Das Substrat nach Anspruch 1, worin das Polysäurepolymer Polyasparaginsäure ist.

5. Das Substrat nach Anspruch 1, worin das Polysäurepolymer Polyglutaminsäure ist.

6. Das Substrat nach Anspruch 1, worin das Polysäurepolymer quervernetzt ist.

Revendications

1. Support pour dispositif médical, avec une surface compatible avec le sang et possédant une substance physiologiquement active présentant un effet d'inhibition de la formation de caillots de sang ou est capable de désagréger des caillots formés, immobilisée sur la surface par l'intermédiaire d'un groupe fonctionnel ou d'un composé pouvant jouer le rôle d'un écarteur, liée à la surface par des liens covalents, caractérisé en ce que ledit composé est un polymère polyacide possédant comme groupes fonctionnels de nombreux groupes carboxyliques auxquels ladite substance physiologiquement active est directement ou indirectement liée, ledit polymère polyacide étant lié par des liaisons covalentes à la surface nue du support.

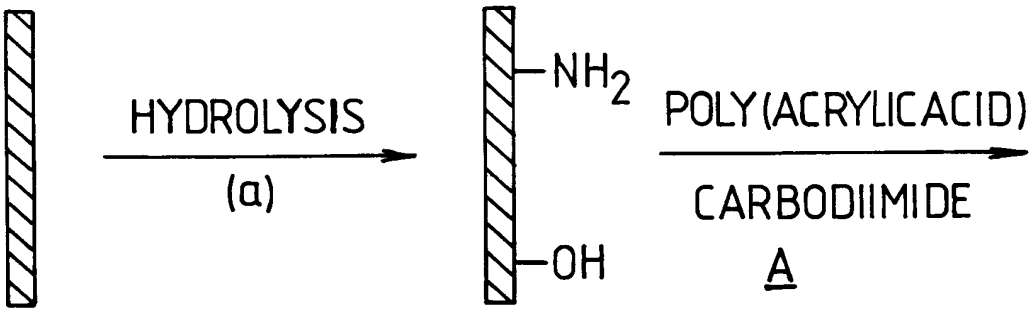
2. Support selon la revendication 1, dans lequel ledit polymère polyacide est un acide polyacrylique ou un acide polyméthacrylique.

3. Support selon la revendication 2, dans lequel ledit polymère polyacide est un polymère comprenant entre 1000 et 10.000 unités de monomère.

4. Support selon la revendication 1, dans lequel le dit polymère polyacide est l'acide polyaspartique.

5. Support selon la revendication 1, dans lequel le dit polymère polyacide est l'acide polyglutamique.

6. Support selon la revendication 1, dans lequel ledit polymère polyacide est réticulé.


HYDROLYSIS
(a)
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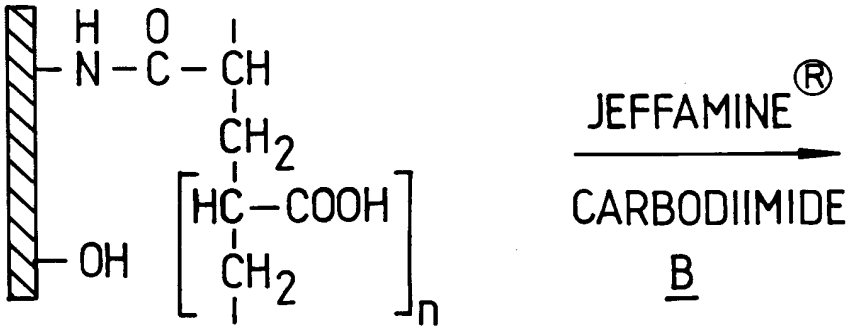
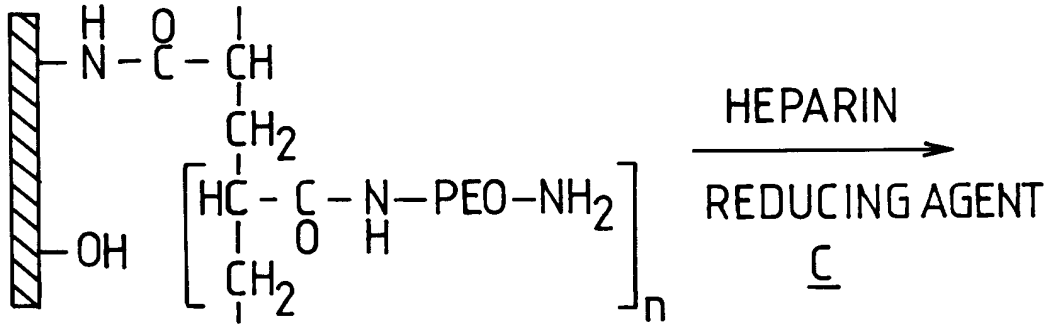
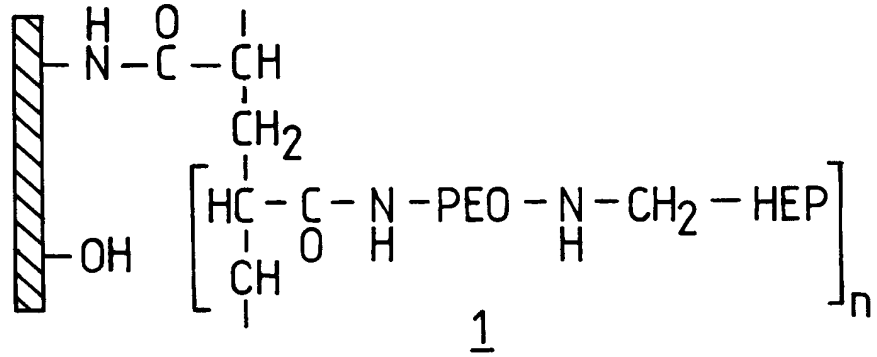
OH
POLY(ACRYLICACID)
CARBODIIMIDE
A


JEFFAMINE®
CARBODIIMIDE
B


HEPARIN
REDUCING AGENT
C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